Daphnia as keystone predators: effects on phytoplankton diversity and grazing resistance

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Theory predicts that a predator can promote coexistence among competing prey, and so enhance prey diversity (the keystone predation effect), by fostering dominance of slow-growing, consumption-resistant prey. In contrast, if the predator promotes dominance by fast-growing vulnerable prey, theory predicts that the predator is unlikely to promote prey diversity. Theory is silent about keystone predation effects when the predator does not cause a net change in the vulnerability of the prey assemblage. I present experimental evidence that Daphnia can act as a keystone predator without causing a net change in the grazing resistance of the phytoplankton assemblage. No change in resistance was observed, despite strong Daphnia effects on the species composition of the phytoplankton.

INTRODUCTION

Keystone predation is a central concept in ecology, despite the term having widely different meanings in different settings (Mills *et al.*, 1993; Menge *et al.*, 1994). One simple definition that bridges the gap between theory and experiment is that a keystone predator promotes coexistence among competing prey. Using this definition, a keystone predator can be identified with an experiment in which the response of prey diversity to predator manipulation is measured. Many such experiments have been performed, and many species can be described as keystone predators as a result (see reviews in Leibold, 1996; Proulx and Mazumder, 1998; Chase *et al.*, 2000). For example, experimental studies have shown that *Daphnia* grazing can enhance phytoplankton diversity (McCauley and Briand, 1979; Beisner, 2001), and consequently that *Daphnia* can act as a keystone predator. In these studies, *Daphnia*'s promotion of phytoplankton diversity was accompanied by a shift in dominance of the phytoplankton assemblage toward species that are more resistant to grazing.

In contrast to the above, the clear-water phase in eutrophic lakes, which is typically driven by high *Daphnia* grazing (Lampert *et al.*, 1986; Sarnelle, 1993), is commonly characterized by relatively low phytoplankton diversity (Fott *et al.*, 1974; Sarnelle, 1993). Indeed, the clear-water phase in eutrophic lakes is somewhat paradoxical in that the phytoplankton assemblage is often dominated by cryptophytes (Sommer, 1985, 1987; Lampert *et al.*, 1986), which are among the most vulnerable taxa with respect to *Daphnia* grazing (Porter, 1977; Sterner, 1989). Paradoxical dominance by cryptophytes during periods of intense grazing has been attributed to high maximum growth rates of these taxa, but direct evidence of the latter is lacking. Taken together, the above observations suggest that phytoplankton species may dominate under strong grazing pressure either by being resistant to grazing mortality or by having high resource-saturated growth rates.

Recent theory on keystone predation provides a framework for understanding the complex interactions described above. In situations where increased grazing fosters dominance by grazing-resistant species, theory predicts that grazing will increase diversity (Holt *et al.*, 1994; Leibold, 1996; Chase *et al.*, 2000), as seen in previous *Daphnia* experiments. In contrast, when increased grazing fosters dominance by vulnerable species with high growth rates ('grazing tolerant' species), as during the clear-water phase, theory predicts that grazing will not increase diversity (Chase *et al.*, 2000). However, theory is silent about the third possibility when increased grazing has no net effect on the relative dominance of resistant versus vulnerable phytoplankton species.

In this article, I examine whether *Daphnia* grazing promotes phytoplankton diversity in a highly eutrophic lake with a large-scale direct manipulation of *Daphnia*. Previous direct manipulations of *Daphnia* that address this question were performed in low-nutrient lakes (McCauley and Briand, 1979; Beisner, 2001). Direct *Daphnia* manipulation (rather than via manipulation of zooplanktivorous fish) obviates mechanistic uncertainties associated with the indirect effects of enclosed fish on phytoplankton assemblage structure via nutrient excretion (Schindler, 1992; Vanni and Layne, 1997; Attayde and Hansson, 1999) that may confound the interpretation of *Daphnia* effects. A gradient design was employed to test a specific prediction of keystone predation theory, namely that phytoplankton diversity may show a unimodal response to a gradient in *Daphnia* (Leibold, 1996). Very few gradient-style experiments exist that examine this prediction (Lubchenco, 1978; Proulx and Mazumder, 1998).

The experiment was also used to examine whether increased *Daphnia* grazing shifted dominance of the phytoplankton toward more or less grazing-resistant species (or had no effect), as a test of the theoretical predictions described above. Grazing resistance was determined via contemporaneous direct measurement of species-specific phytoplankton resistance to *Daphnia* grazing. Thus, grazing resistance was measured in the exact context of the *Daphnia* manipulation, a unique advantage of this study. The results show that *Daphnia* can promote phytoplankton species evenness without changing the overall grazing-resistance of the phytoplankton assemblage.

METHODS

The experiments were conducted in Zaca Lake (34.778° N, 120.039° W), a naturally eutrophic lake in southern California, USA. The lake is thermally stratified for much of the year, leading to persistent anoxia in the deep waters. Total phosphorus (dissolved plus particulate) concentrations in the mixed layer average about 10 μM during winter mixing periods and range from 2.5 to 8.1 μM during stratified periods, making the lake highly eutrophic (Sarnelle, 1992).

An enclosure experiment was conducted in Zaca Lake during April–May of 1994 to assess the effect of *Daphnia* on phytoplankton diversity and species composition. The experimental methods have been described previously (Sarnelle, 2003) and are briefly summarized here. Twelve tubular polyethylene enclosures (diameter, 2.3 m; depth, 8 m) were deployed in the middle of Zaca Lake on 17 April 1994 and sampled until 21 May 1994. Enclosures were manipulated by slowly pulling a large zooplankton net (diameter, 1 m; mesh, 250 μm) through each enclosure every 3–4 days. The same number of net hauls was made in every enclosure to control for mixing effects, whereas the gradient of *Daphnia* removal was established by varying the number of times the contents of each net haul was either removed from the enclosure (ranging from 0 to 13 times per week) or emptied back into the enclosure (sham hauls, no zooplankton removed). Zooplankton collected in sham hauls were released back into the enclosure without lifting the net out of the water.

Enclosures were sampled twice weekly for phytoplankton and zooplankton and once a week for soluble reactive phosphorus (SRP) and NH_4^+. Transparency was measured weekly with a 20-cm Secchi Disk. Depth-integrated samples for phytoplankton were taken from the euphotic zone (surface to depth of 80% dissolved oxygen saturation) with a tube sampler (51 mm inside diameter). Phytoplankton were preserved in Lugol's solution and enumerated via the inverted microscope technique (Sarnelle, 1993, 2003). The dry biomass ($\mu g \ L^{-1}$) of each species was calculated from estimates of average cell volume, assuming that phytoplankton have a specific gravity of 1 and a dry : wet mass ratio of 0.10 (Rieman and Falter, 1981). To assess *Daphnia's* effects on phytoplankton assemblage structure, relative abundance data for the 10 most common species were subjected to principle components analysis (PCA) and the resulting PCA factor scores were used as response variables in statistical tests.

Consumer effects on prey species diversity may be manifest via effects on the number of prey species (richness) or the distribution of biomass among prey species (evenness). For example, models typically predict a shift in the prey community from species 'A' by itself at low predator density to species 'A' and 'B' coexisting at moderate predator densities to species 'B' by itself at high predator densities. These shifts represent changes in prey richness from one to two to one across the predation gradient but also changes in the distribution of biomass (evenness) from 100% species 'A' to a 50:50 mixture and then to 100% species 'B'. Thus, it is reasonable to assess keystone predation effects using richness or evenness as the metric of species diversity (Hurlbert, 1971). Although both diversity components were calculated in this study, I rely on evenness metrics for practical reasons. Firstly, changes in richness via complete exclusion (at either end of the predator gradient) will require more time than changes in evenness since declining species should become rare before they become totally extinct. Thus, evenness should respond more rapidly to consumer manipulation than richness and so should be more sensitive to the experimental manipulation. Secondly, richness measures are affected by the highly variable detection of species that are always rare, which in phytoplankton assemblages may represent 'accidentals', i.e., species that recruit to and disappear from the pelagic zone without

necessarily being dynamically linked to other planktonic species (Hutchinson, 1961). Lastly, estimates of species richness are strongly influenced by the number of individuals enumerated (Magurran, 1988), a factor that was not held constant across samples in this study. In contrast, the metrics I used to estimate species evenness are insensitive to this potential artifact (see next paragraph). Thus, the species evenness estimates employed in this study should be more sensitive to experimental manipulations and have lower residual variance than species richness estimates. Given these considerations, use of a species diversity index that combines richness and evenness is likely to be less sensitive and precise than an index of evenness alone.

Phytoplankton evenness was quantified using the Species Dominance Index, which is simply the relative abundance (as proportion of total phytoplankton biomass) of the most abundant species multiplied by −1 (Berger and Parker, 1970; Magurran, 1988), and the Lorenz Equitability Index (Taillie, 1979; McAuliffe, 1984) as applied to the 10 most common species (which accounted for 87% of total phytoplankton biomass, on average). These indices were chosen because they are mathematically independent of species richness and insensitive to the problematic detection of rare species and so virtually immune to artifacts related to the number of individuals counted. The Lorenz Index is two times the area beneath the curve (Lorenz Curve) that describes the relationship between the cumulative proportion of species and the cumulative proportion of biomass in an assemblage (McAuliffe, 1984). In a perfectly even assemblage (Index = 1), the Lorenz Curve is a diagonal line that bisects the area of a graph having axes ranging from zero to one.

Phytoplankton response variables were averaged over the last three sampling dates of the experiment (days 25, 28, 32) for all analyses, since the results are being compared to theoretical predictions at equilibrium (Sarnelle, 2003). To assess whether phytoplankton biomass or assemblage structure was reasonably stable over the sampling window, I performed repeated-measures analyses of variance (ANOVAs) on total biomass and PCA factor scores. These analyses revealed no time or time–treatment interactions (Wilks' Lambda statistic, $P > 0.13$ for total biomass, $P > 0.50$ for PCA scores), indicating that there were no significant changes in total biomass or phytoplankton assemblage structure over the 7-day sampling window.

Crustacean zooplankton were sampled with 8-m vertical hauls of a 13-cm diameter, 102-μm mesh net, which was assumed to have an efficiency of 60% based on previous empirical experience (Sarnelle, 1992). Four net hauls from each enclosure were pooled into a single sample, and samples were preserved in sucrose-formalin (Haney and Hall, 1973). Zooplankton were counted and measured at ×40 in a Sedgwick-Rafter cell. Measurements of body length were made with a digitizer and drawing tube and used to calculate dry biomass (μg L^{-1}) using equations developed from dried Zaca Lake specimens. *Daphnia* biomass was averaged over the last four sampling dates of the experiment (days 21, 25, 28, 32) to examine the effect of the removal manipulation on *Daphnia* and the effect of *Daphnia* on phytoplankton abundance, evenness and assemblage structure. The results were not sensitive to the exact combination of dates included in the observation window. Enclosure results were analysed via linear and polynomial regression. Log transformation was applied as needed to satisfy the assumptions of parametric statistical analysis.

The enclosure experiment was designed to measure the net effect of *Daphnia* on the phytoplankton assemblage under near natural conditions and potentially included both direct negative (via consumption) effects and indirect positive effects stemming from grazer excretion of dissolved nutrients (N and P), decreased algal uptake of nutrients, and increased light availability (Sterner, 1986; Spencer and King, 1987; Elser, 1992; Vanni and Layne, 1997). To isolate direct mortality effects and so obtain independent estimates of the relative grazing resistance of individual phytoplankton taxa, a short-term *Daphnia* manipulation was conducted on May 10 and 11 (days 19–20 of the enclosure experiment). This experiment was conducted in the dark to prevent the phytoplankton from showing growth stimulation in response to grazer presence (Sarnelle, 1993).

Phytoplankton were collected from the lake and from 6 of the large enclosures having widely divergent *Daphnia* densities, screened through 48-μm mesh (which effectively removed all but protozoan grazers) and pooled. The large enclosures developed contrasting algal communities in response to *Daphnia* manipulation, so mortality rates of many species could be determined simultaneously in a single incubation via this pooling technique. To maximize *Daphnia*'s filtering rates and reduce the impact of grazing by ciliates (Landry and Hassett, 1982), the pooled phytoplankton were diluted (∼50:50) with filtered lake water (1-μm nominal retention). Ciliate densities (*Strombidium*, *Strobilidium*) were very low after dilution (290–530 individuals L^{-1}). Ciliate clearance rates on small phytoplankton are on the order of ∼2 μL individual^{-1} h^{-1} (Jürgens *et al.*, 1996), so these densities translate into negligible grazing rates by the ciliate populations in the 24-h mortality experiment (14–25 mL day^{-1}, or 1–2% over the incubation).

Daphnia were collected from a high-*Daphnia* enclosure, concentrated with a 250-μm mesh sieve (to reduce densities of other crustaceans) and added to the diluted

phytoplankton. The phytoplankton–*Daphnia* mixture was then dispensed into buckets. Two treatments were applied by sieving the water in three of the buckets through 250-μm mesh (control) while leaving four buckets unsieved (grazed). The contents of each bucket were then sampled for initial phytoplankton densities, poured into a 10 L polyethylene container (cubitainer) and incubated in the lake for 24 h. Cubitainers were enclosed in a double layer of black plastic during incubation, which decreased light availability by 99.99%.

At the end of the incubation, each cubitainer was sampled for final densities of phytoplankton and zooplankton. Instantaneous mortality rates (day^{-1}) for each phytoplankton species were determined as: $\ln(C_i)$–$\ln(C_f)$, where C_i and C_f are initial and final phytoplankton cell densities, respectively. Mortality rates were corrected for changes in the controls, which were typically less than $\pm 25\%$. Mortality rates were standardized by expressing each species' mortality as a proportion of the highest mortality rate in the data set (Vanderploeg and Scavia, 1979). One species, *Oocystis sp.*, tended to increase in controls, so mortality rates calculated for this species could be suspect. However, inclusion of this species in subsequent analyses did not impact any results.

Statistical tests of the hypothesis that the phytoplankton species in Zaca Lake differed in their resistance to *Daphnia* grazing necessitated a separate analysis from the one described above. Mortality rates of different taxa in the same experiment cannot be directly compared statistically, because the rates as calculated above are not independent (Sarnelle, 1997). To circumvent this problem, I tested the analogous hypothesis that the magnitude of *Daphnia's* grazing effect differed across phytoplankton species in the short-term *Daphnia* manipulation as follows. Independent comparisons of *Daphnia's* effect for any two phytoplankton species can be generated by considering a commonly used index of effect size: the magnitude of *Daphnia's* predatory impact (PI) for each species can be estimated as $PI = -\left[\left(\overline{\ln C_c}\right) - \left(\overline{\ln C_g}\right)\right]$, where $\overline{\ln C_c}$, $\overline{\ln C_g}$ are the means of natural log-transformed cell densities in control and grazed replicates, respectively (Cooper *et al.*, 1990). A statistical test of the difference between PIs for two species within an experiment (PI_1–PI_2) is mathematically equivalent to a comparison of the difference between the means of the natural log-transformed ratios of cell densities of the two taxa in each treatment (see Sarnelle, 1997 for derivation). A ratio of cell densities for any two species can be calculated for each independent experimental replicate, permitting valid statistical tests. Thus, to test whether two species of phytoplankton varied in their resistance to *Daphnia* predation in the short-term *Daphnia* manipulation, I compared means of natural log-transformed ratios

of final cell densities between control and grazed treatments using two-tailed *t*-tests that allowed for unequal variances.

RESULTS

The removal manipulation was successful in creating a wide gradient of *Daphnia* biomass across the enclosures without affecting any other zooplankton taxa significantly (Fig. 1). Rotifer biomass was always negligible. Although other crustacean zooplankton taxa were abundant in a few of the enclosures, none were affected by the manipulation (Fig. 1). This does not mean that other crustacean taxa were not captured in the removal net, but rather that the mortality inflicted on such taxa by the
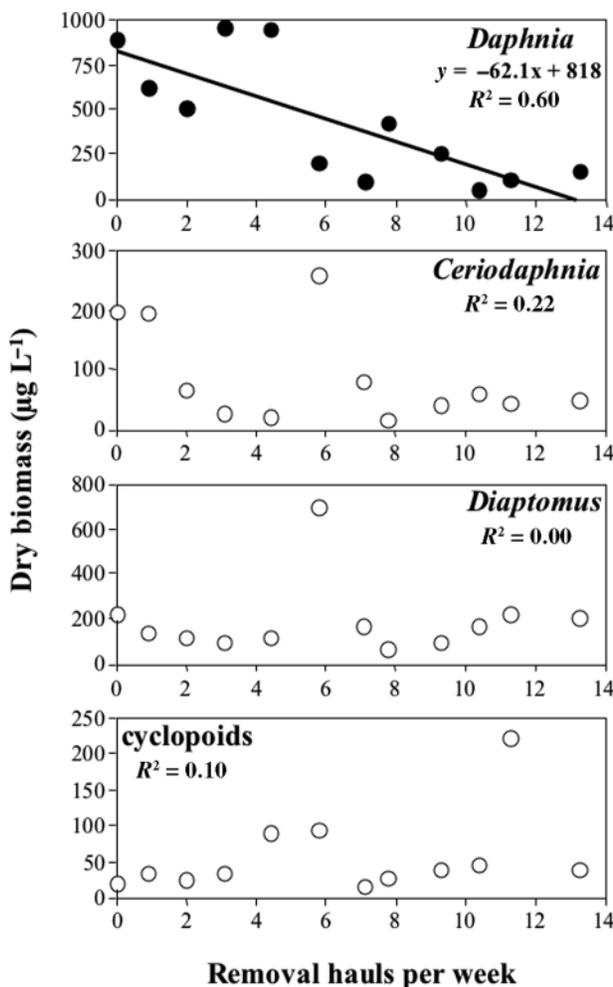

Fig. 1. Effect of the removal manipulation on the dry biomass of *Daphnia* (upper panel, linear regression, analysis of variance (ANOVA) *F*-test, $P < 0.005$) and other crustacean zooplankton (ANOVA *F*-tests, $P > 0.10$) in the enclosure experiment. Data are averages of days 21, 25, 28 and 32.

net was small relative to birth rates, the latter potentially being elevated in response to strong *Daphnia* removal. Attempts to relate phytoplankton response variables to total herbivore biomass (*Daphnia* + *Ceriodaphnia* + *Diaptomus*) or total crustacean biomass (herbivores + cyclopoids), rather than to *Daphnia* biomass alone, resulted in poorer fits, suggesting that other herbivores did not have a major modifying influence on the responses of interest in this article. This does not mean that these other taxa had no influence on the phytoplankton but rather that their influence was likely limited to introducing scatter into the relationships between *Daphnia* biomass and phytoplankton response variables.

Daphnia pulicaria was the dominant daphnid species in the experiment, averaging ~80% of total *Daphnia* biomass over all but one enclosure, and there was no effect of the removal gradient on the distribution of biomass among the daphnid species present (linear regression, $P > 0.50$). In one enclosure (5.8 removals per week), *Daphnia* biomass comprised a mixture of three species (*D. pulicaria*, 26%; *D. galeata*, 29%; *D. parvula*, 45%), but this enclosure was not found to be exceptional in any subsequent analysis. Maximum *Daphnia* biomass in the enclosures was ~1000 µg dry mass L^{-1} (Fig. 1), a value that is nearly identical to maximum *Daphnia* biomass observed in the lake when zooplanktivorous fish were rare (Sarnelle, 1997). Mean individual *Daphnia* dry mass varied from 7 to 38 µg across the enclosures and was positively related to *Daphnia* biomass (Fig. 2). The latter relationship was significantly curvilinear, with individual mass increasing with biomass up to ~300 µg L^{-1} and then leveling off (Fig. 2).

Daphnia had a strong negative effect on total phytoplankton biomass (Fig. 2). There was a significant positive effect of *Daphnia* on phytoplankton evenness as measured by the Species Dominance Index (linear regression, ANOVA *F*-test, $P < 0.05$, Fig. 2), and a nearly significant positive effect on the Lorenz Equitability Index (linear regression, ANOVA *F*-test, $P < 0.06$; Fig. 2). There was no statistical evidence of non-linearity in the evenness responses. Species richness was not affected by *Daphnia*, but polynomial regression analysis identified two enclosures as substantial outliers (studentized residuals = 3). Omitting these outliers (the two highest richness estimates), a significant unimodal relationship between richness and *Daphnia* biomass was obtained ($y = 0.004x - 0.00001x^2 - 0.53$, $R^2 = 0.79$, $P < 0.05$ for significance of linear and quadratic terms). However, the conclusion that *Daphnia* promoted phytoplankton diversity in the experiment is solely based on the evenness results (Fig. 2). Phytoplankton assemblage structure showed strong dominance by *Coelastrum microporum* at low *Daphnia* biomass (<250 µg L^{-1}) and a shift to a more equitable distribution of biomass above this
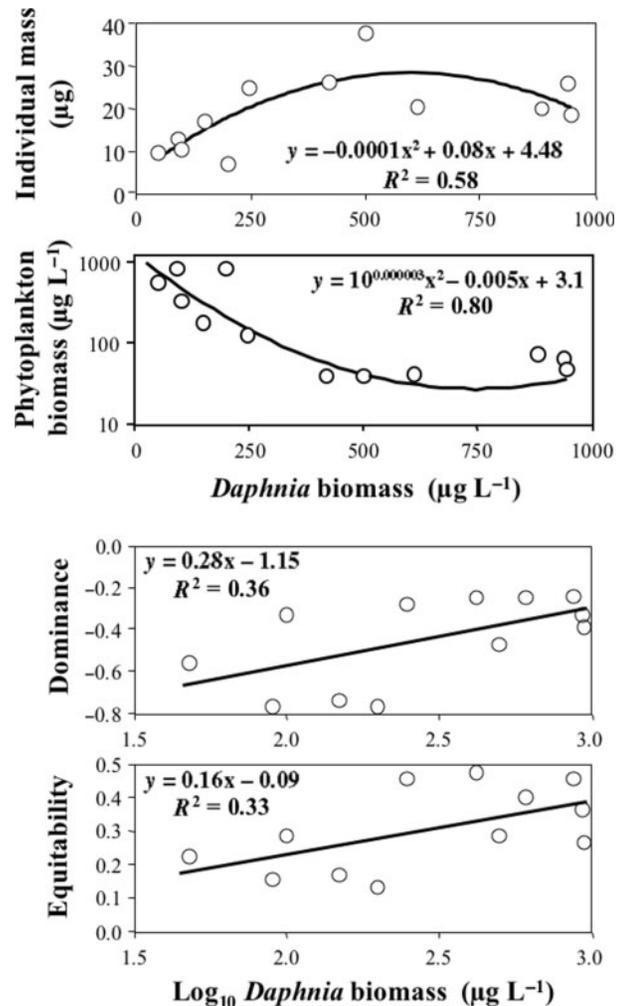

Fig. 2. Relationship between *Daphnia* biomass and individual *Daphnia* dry mass [polynomial regression, analysis of variance (ANOVA) *F*-test, $P < 0.03$], and the effects of *Daphnia* on total phytoplankton dry biomass [polynomial regression, analysis of variance (ANOVA) *F*-test, $P < 0.001$], and phytoplankton species evenness as measured by the Species Dominance Index (linear regression, ANOVA *F*-test, $P < 0.05$) and the Lorenz Equitability Index (linear regression, ANOVA *F*-test, $P < 0.06$) in the enclosure experiment. Phytoplankton data are averages of days 25, 28 and 32.

level (Fig. 3), a pattern that is consistent with the positive effect of *Daphnia* on phytoplankton evenness (Fig. 2).
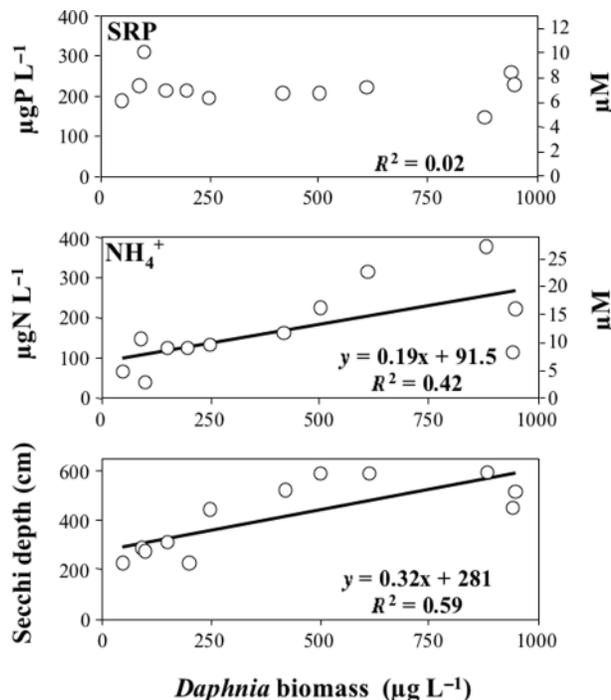
Concentrations of NH_4^+ in the enclosures were positively related to *Daphnia* biomass (linear regression, ANOVA *F*-test, $P < 0.03$), but SRP concentrations were not (linear regression, ANOVA *F*-test, $P > 0.60$, Fig. 4). Concentrations of SRP were at saturating levels in all the enclosures (Fig. 4). Light penetration (as Secchi Depth) was also positively related to *Daphnia* biomass (linear regression, ANOVA *F*-test, $P < 0.01$, Fig. 4).

The results of the short-term mortality experiment indicated that the phytoplankton species in Zaca Lake
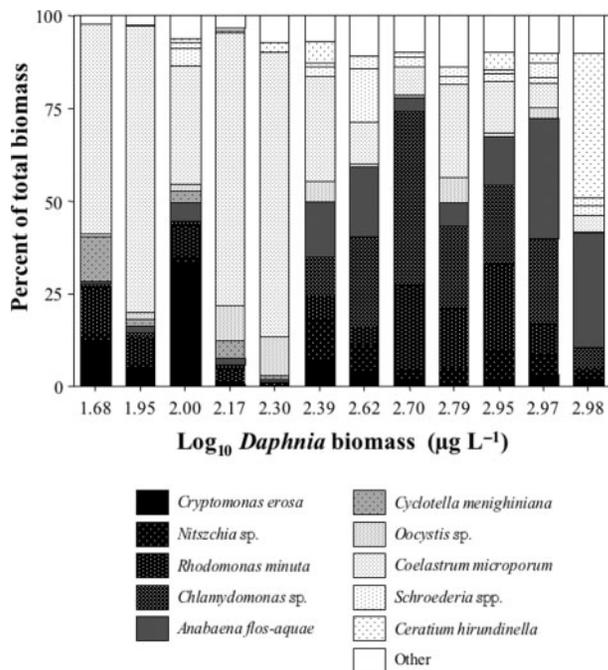
Fig. 3. Relative abundances of the common phytoplankton species in the enclosure experiment (averages over days 25, 28 and 32). Columns are arranged in rank order from lowest to highest *Daphnia* biomass. Phytoplankton species are arranged in rank order from lowest (darkest fill, bottom of stack) to highest (lightest fill, top of stack) resistance to *Daphnia* grazing, based on mortality data in Table I. Data are averages of days 25, 28 and 32.

Fig. 4. The effects of *Daphnia* on SRP [linear regression, analysis of variance (ANOVA) *F*-test, $P > 0.60$] and NH_4^+ (linear regression, ANOVA *F*-test, $P < 0.05$) concentrations, and Secchi Depth (linear regression, ANOVA *F*-test, $P < 0.01$) in the enclosure experiment. Data are averages of days 21 and 28.

varied strongly in their resistance to *Daphnia* predation. As expected from previous studies (Porter, 1977; Sterner, 1989), small diatoms (*Nitzschia*, *Cyclotella*) and cryptophyte flagellates (*Cryptomonas*, *Rhodomonas*) were the most vulnerable taxa (Table I). The most resistant taxa were chlorophytes (*Coelastrum*, *Oocystis*, *Schroederia*), of which one species (*Coelastrum*) was observed to form colonies up to 45 μm in diameter in the large enclosures. *Coelastrum* was also, on average, the most dominant species across the large enclosures (Table I). The two most resistant species (*Schroederia*) were of edible size, suggesting that these species resist digestion in *Daphnia*'s gut (Porter, 1977), but these species were generally rare in the enclosures (Fig. 3). Statistical tests showed that there were highly significant differences among the phytoplankton species in their resistance to *Daphnia* grazing. For example, the mortality effect of *Daphnia* on *Cryptomonas* was significantly larger than on *Anabaena* ($P < 0.03$) and all other species with mortality rates lower than *Anabaena* ($P < 0.02$). Similarly, the mortality effect of *Daphnia* on *Schroederia judayi* was significantly smaller than on *Oocystis* ($P < 0.01$) and all other species with mortality rates higher than *Oocystis* ($P < 0.01$).

To address the question of whether phytoplankton assemblage structure shifted to being more resistant to *Daphnia* grazing at high *Daphnia* densities in the enclosure experiment, I calculated a vulnerability index for each enclosure assemblage based on the standardized mortality rates and relative abundances of each species in each enclosure (Table I). The vulnerability score for each enclosure was calculated as M_iP_i, where M_i is the standardized mortality rate and P_i is the relative abundance (as percentage of total phytoplankton biomass) of species i. The maximum possible value of the community vulnerability index is 100, corresponding to a monoculture of *Cryptomonas erosa*, the most vulnerable species. Of the species listed in Table I, *Coelastrum microporum*, *Anabaena flos aquae*, *Rhodomonas minuta* and *Cryptomonas erosa* accounted for an average of 61% of phytoplankton biomass in the enclosures. *Chlamydomonas sp.* accounted for an additional 13.2% of average biomass across the enclosures but was too rare during the mortality experiment to obtain an estimate of its resistance to *Daphnia* grazing. Consequently, I estimated overall assemblage vulnerability in the enclosure experiment in two ways: excluding *Chlamydomonas* from the data set, and including *Chlamydomonas* by assigning it a relative mortality equal to that for *Rhodomonas*, a similarly sized flagellate (5–10 μm). The same conclusions were reached regardless

Table I: Mortality rates (day^{-1}) inflicted by Daphnia grazing on coexisting phytoplankton species (as measured via 24-h dark incubation) and phytoplankton assemblage structure in the long-term enclosure experiment

Species	Mortality experiment		Enclosure experiment		PCA factor 1 correlation
	Mortality (SE)	Standardized mortality	Percent biomass		
			Mean	Range	
Coelastrum microporum	0.40 (0.41)	0.12	34.4	4.2–77.0	−0.94
Chlamydomonas sp.	ND	0.67[a]	13.2	0.4–47.0	0.76
Anabaena flos-aquae	1.68 (0.48)	0.51	10.8	0.5–32.5	0.74
Rhodomonas minuta	2.18 (0.35)	0.67	9.7	0.6–23.7	0.32
Cryptomonas erosa	3.26 (0.60)	1.00	5.8	0.2–32.8	−0.18
Nitzschia sp.	2.31 (0.31)	0.71	3.8	0.2–10.9	0.64
Oocystis sp.	0.92 (0.39)	0.28	3.5	0.2–10.2	−0.53
Schroederia setigera	0.07 (0.27)	0.02	2.8	0.1–14.5	0.57
Cyclotella menighiniana	1.32 (0.35)	0.40	2.0	0.0–12.1	−0.64
Schroederia judayi	0.05 (0.42)	0.01	1.5	0.1–4.2	0.86

ND, not detected; PCA, Principle Components Analysis; SE, standard error. Mortality rates were standardized relative to the mortality of *Cryptomonas erosa*, the species that was the most vulnerable to grazing. Assemblage structure is given as percent biomass and as correlations between each species' relative biomass and factor scores from PCA (see text for details). Correlations with absolute values >0.6 were statistically significant at $P < 0.05$ (uncorrected probabilities). Mortality data from Sarnelle (2003).
[a]Relative mortality assumed to be equivalent to *Rhodomonas minuta*.

of which method was used, so I only present results for the second method. Although the relative mortalities of the phytoplankton species in Zaca Lake varied over two orders of magnitude (0.016–1), and the overall vulnerability of the phytoplankton assemblage varied substantially across the large enclosures (14–53), the *Daphnia* gradient had no effect on the overall vulnerability of the phytoplankton community (linear and polynomial regressions, ANOVA *F*-tests: $P > 0.30$, Fig. 5).

PCA of phytoplankton assemblage structure resulted in a large fraction of the total variance (43%) being accounted for by a single factor (factor 1). *Daphnia* had a significant positive affect on scores of this factor (Fig. 5), indicating that *Daphnia* had a significant effect on assemblage structure. Given the pattern of species correlations with factor 1 scores (TableI), *Daphnia's* positive effect on factor 1 scores suggested that the phytoplankton assemblage shifted away from dominance by *Coelastrum*, one of the most resistant species, and toward dominance by *Chlamydomonas* and *Anabaena*, which were moderately vulnerable to grazing. This result is consistent with the patterns in assemblage structure (Fig. 3) and was confirmed by analyses of the individual responses of these three species—the relative abundance of all three species responded significantly, and in the direction expected based on the PCA, with respect to the *Daphnia* gradient (linear regressions, ANOVA *F*-tests, $P < 0.01$). Notably, the

relative abundance of *Cryptomonas erosa*, the most vulnerable phytoplankton species, was not affected by the *Daphnia* gradient (linear regression, ANOVA *F*-test, $P > 0.15$).
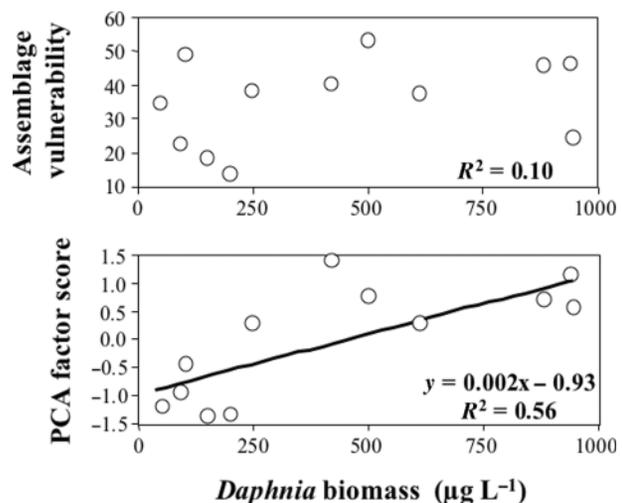

Fig. 5. The effect of *Daphnia* on the overall grazing vulnerability of the phytoplankton assemblage [upper panel, linear and polynomial regression, analysis of variance (ANOVA) *F*-tests, P > 0.30], and factor 1 scores from principle components analysis (PCA) of phytoplankton relative abundance (lower panel, linear regression, ANOVA *F*-test, P < 0.005). Data are averages of days 25, 28 and 32.

DISCUSSION

The enclosure results (Fig. 2) echo those of two previous experiments in low-nutrient lakes (McCauley and Briand, 1979; Beisner, 2001) showing that *Daphnia* can function as a keystone predator. This is the first such experimental result from eutrophic lakes. Other experimental studies suggestive of a positive effect of *Daphnia* on phytoplankton diversity employed fish manipulations (see review by Proulx and Mazumder, 1998), which confound the impacts of *Daphnia* with the impacts of fish excretion on phytoplankton assemblage structure (Schindler, 1992; Vanni and Layne, 1997; Attayde and Hansson, 1999).

The mortality results showed that *Daphnia*, despite its generally non-selective feeding behavior, functions as a highly-selective mortality agent with respect to the phytoplankton assemblage (Table I). This result is consistent with previous studies (Porter, 1977; Lehman and Sandgren, 1985; Elser, 1992; Sarnelle, 1993; Epp, 1996). However, *Daphnia* did not shift the phytoplankton assemblage toward greater overall dominance by resistant species in the experiment (Fig. 5), despite strong effects on phytoplankton species composition (Figs 3 and 5). Two species with moderate vulnerabilities (*Anabaena* and *Chlamydomonas*) were favored by increased *Daphnia* while one species with low vulnerability (*Coelastrum*) was not. As a consequence, the experimental results in this article are unique and not encompassed by existing elaborations of keystone predation theory, since the case where the predator does not alter overall consumption-resistance of the prey assemblage has not been analysed (Leibold, 1996; Chase *et al.*, 2000).

Theory does allow that high levels of selective mortality may drive phytoplankton assemblages toward dominance by species that are highly vulnerable to grazing (Armstrong, 1979), as seen during clear-water phases in eutrophic lakes. Clear-water phases dominated by highly vulnerable cryptophytes can be explained if phytoplankton species trade the ability to resist grazing against the ability to grow fast at saturating resource levels Chase *et al.*, 2000). Meta-analysis of grazing resistance and maximum growth rate provides some support for the existence of this tradeoff for phytoplankton (Agrawal, 1998). The enclosure results indicate that phytoplankton resources were likely saturating in enclosures with high levels of *Daphnia* biomass (NH4$^+$, light, Fig. 4), or in all the enclosures (SRP, Fig. 4), as they are during the clear-water phase in Zaca Lake (Sarnelle, 1992). This counterintuitive effect of herbivory should be more likely in high-productivity systems where prey resources are more likely to be at saturating levels (and so maximal growth rates more critical in determining prey species success) when herbivory is intense.

The enclosure results provided no clear evidence of a unimodal response of phytoplankton evenness to the *Daphnia* gradient, which may be related to the lack of change in phytoplankton assemblage resistance. In theory, unimodal diversity responses are expected when the prey assemblage shifts from dominance by vulnerable species at low predator densities to a mixture of vulnerable and resistant species at moderate predator densities and then to dominance by resistant species at high predator densities (Leibold, 1996). In the enclosure experiment, the phytoplankton assemblage shifted from dominance by a highly resistant species (*Coelastrum*) at low *Daphnia* density to a mixture of vulnerable and resistant species at moderate *Daphnia* density but did not shift further at high *Daphnia* density (note leveling off of PCA scores above ∼400 µg L^{-1} *Daphnia* biomass in Fig. 5). One possible explanation for this asymptotic behavior may be that *per capita* grazing effort by *Daphnia* declined at the lowest food densities (Plath, 1998; Plath and Boersma, 2001). Thus, although *Daphnia* biomass changed linearly across the experimental gradient (Fig. 1), total grazing by the *Daphnia* population may have approached an asymptote at higher *Daphnia* densities because of reduced *per capita* grazing. It is also possible that the experiment did not last long enough for phytoplankton assemblage structure to fully equilibrate to the *Daphnia* gradient.

The experimental results were somewhat surprising because previous experiments and observations in Zaca Lake suggested that *Daphnia* grazing favors dominance by cryptophytes during clear-water phases, which typically occur in April (Sarnelle, 1993). Cryptophytes (*Cryptomonas erosa* and *Rhodomonas minuta*) were only two of several dominant species in the experiment, and the relative abundance of cryptophytes was not affected by the *Daphnia* manipulation. One potential explanation for the lack of cryptophyte success in the experiment is the degree of mixing induced by the *Daphnia* removal manipulation. Although mixing was held constant across the enclosures, the degree of mixing in the enclosures was greater than in the lake, as judged by the steepness of oxygen versus depth gradients (Sarnelle, unpublished data). Flagellates appear to be generally less successful under mixed conditions (Reynolds, 1984), perhaps because the advantages of vertical motility are reduced when the water column is less stratified. Thus, increased mixing in the enclosures may have suppressed cryptophyte success relative to the clear-water phase in the lake. This may have had an impact on *Daphnia*'s overall effects on phytoplankton diversity and grazing resistance, but it is not possible to determine the nature of the impact without an experiment in which mixing is manipulated (Beisner, 2001).

In summary, the experiment demonstrated a positive effect of *Daphnia* on phytoplankton diversity in a

eutrophic lake, but this effect was not associated with a shift toward greater grazing resistance among the phytoplankton. Consequently, further elaborations of keystone predation theory should examine the case where there is no response of overall prey assemblage resistance to consumption. The contrasting observation of low phytoplankton diversity and cryptophyte dominance during *Daphnia*-induced clear-water phases (Sommer, 1985; Lampert *et al.*, 1986; Sommer, 1987) may signal that *Daphnia* can function as a keystone or anti-keystone (i.e., diversity reducing) predator in different environmental contexts, a possibility that has been predicted by keystone predation theory, but not yet observed in experiments.

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